Bacterial Transformation Lab: 12/17/15

- **Introduction:**

The purpose of this lab is to successfully perform a transformation protocol, more specifically the NEB 10 β (Beta) High Efficiency Transformation Protocol, and gain a more fluid understanding of how this will apply to future researching endeavors for the Lambert iGEM Team.

Bacterial Transformation serves as an essential basis to a number of DNA technologies. It involves the transfer of genetic information into a cell by the direct uptake of DNA. Transformation can occur naturally, however, the incidence is extremely rare and constricted to very few strains of bacteria. With that in mind, modern advances in biotechnology, more specifically the use of plasmids, enable researchers and synthetic biologists to tranfer genes (such as those for antibiotic resistance) from foreign DNA into recipient bacterial cells (such as E.Coli or Chinese Hamster Ovary Cells (CHO_s)); this can be achieved through the use of restriction enzymes, enzymes that are used to cut and insert pieces of foreign DNA into plasmid vectors.

- **Materials:**

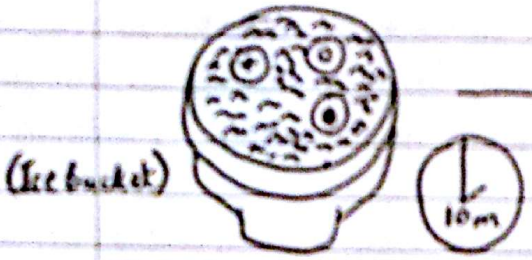
1) Stock of NEB-10 β Competent E.Coli Cells
2) Transformation Tubes
3) Ice Bath / Timer
4) Room Temperature Soc [950mL]
5) PUC-19 Control Plasmid [1-5mL of 1pg - 100ng Plasmid]
6) 42°C Water Bath
7) "Shake 'n' Bake" at 37°C
8) Selection Plates at 37°C
9) Incubator
10) Micropipettes

- **Procedure:** Tranformation protocols can be conceptualized into 4 major steps:

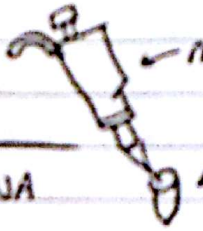
1) Preincubation
2) Incubation
3) Heat Shock
4) Recovery

} —— Detailed Procedure ⟶ (next pg.)

① Thaw a tube of NEB-10 β Competent E. Coli Cells on ice for 10 minutes.



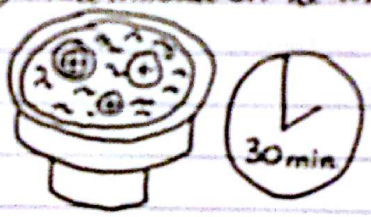
② Carefully pipette 50µl of cells into the transformation tubes on the ice



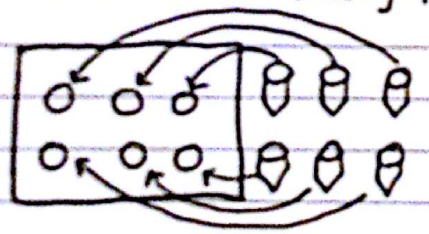
③ Add 1-5µl containing 1 pg - 100 ng of plasmid DNA to a cell mixture.

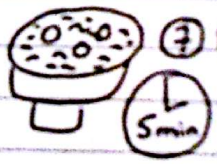
④ Carefully flick the tube 4-5 times to mix cell and DNA. DO NOT VORTEX!

⑤ Place mixture on ice for 30 minutes



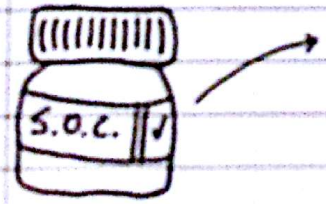
⑥ Heat Shock at exactly 42°C for exactly 30 seconds. Do not mix!



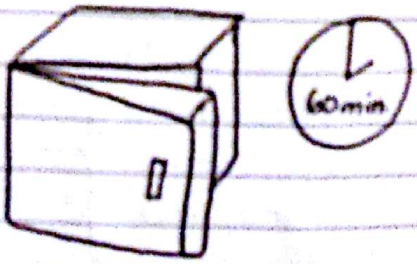


⑦ Place on ice for ~~30~~ 5 min.

⑧ Pipette 950 µl of SOC at room temperature into the mixture



⑨ Place at 37°C for 60 minutes. Shake vigorously (250rpm) or rotate.



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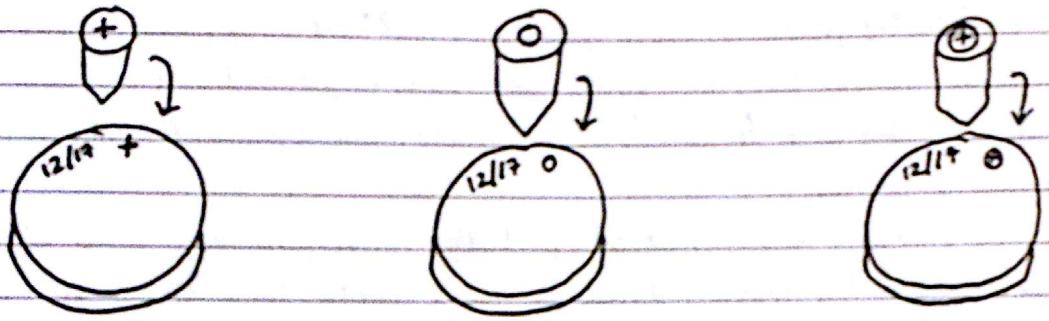
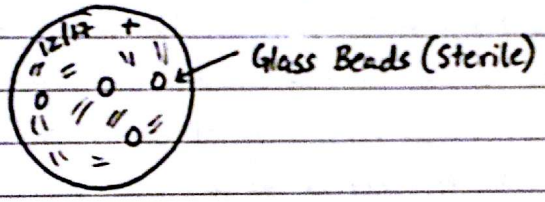
⑩ Warm selection plates to 37°C. Label your plates according to the control variables and then pipette about 15 µl from the transformation tube into the selection agar plates.



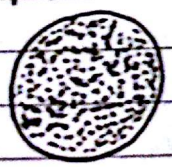
⑪ To spread cells evenly across the plate in an orderly yet quick fashion, add 3-4 sterile glass beads and rotate them in the selection plates until all cells have been distributed evenly.



⑫ After removing glass beads, invert the plates and incubate at 37°C overnight.

- Pre-lab Questions:

1) A successful transformation would have no irregularities and it would successfully express the targeted gene. Also, it would have colonies growing on it.



- Control Variables:

O = No plasmid on ~~[illegible]~~ Amp plate → We expect no growth

+ = Plasmid on plain plate → We expect a whole bunch of ~~[illegible]~~ colonies

⊕ = Plasmid → Amp plate

- Post-Lab ■ Discussion:

In the lab, three variables were defined - (1) ○ = Plasmid less amp plate (2) + = Plasmid on plain plate (3) ⊕ Plasmid on amp plate. Before commencing the procedure, we hypothesized that: the amp plate without a plasmid will have no growth; the plain plate plate with a plasmid will a generous amount of growth; and finally the amp plate with a plasmid will experience a small amount of growth.

The procedure was carried out perfectly. Luckily, no major errors were made in micro pipetting and the experiment was an overall success. Since there were 14 members present, we split into 4 groups, three groups with 4 members each and one with only 2. With that in mind, this division of lab groups helped facilitate a more specialized lab experience, thus allowing us to wholly learn the procedure by heart.

With that in mind approximately 24 hours after the plates final plate were placed into the incubator to allow growth for cell growth, the hypothesis stated before the start of the experiment was accepted. As mentioned before, the amp plate with no plasmid showed no growth whatsoever whereas the plain plate with plasmid showed an abundant growth of bacteria and the amp plate with plasmid showed little or no growth.

- Observations:

Before thawing the tube of NEB-10β Competent E.Coli Cells, we prepared amp plates using "S.O.C." (Super Optimal Broth). This liquid was heated to a high level, and had to be shaken (gently) continuously until 10-15 minutes had passed. During these 10-15 minutes, the SOC gave off a musty smell and when it came time to pour, it we noticed that the SOC had a very high viscosity and had to be dealt with very carefully. Later on, when we began thawing the NEB-10β E.Coli Cells in an ice bath, we saw no color change in the cuvettes - the cells remained completely more or less very colorless. When the plates were completed and placed in the incubator, they were completely odorless; however, approximately 24 hours after the plate were placed in the incubator, a thick, musty, and almost fungal smell reeked from the incubator, indicating that our cells had indeed transformed just as we like we had hoped.

Potential Projects - Brainstorm / Questions: 1/21/16

- **Zinc Biosensor Questions:**

1) Does the biosensor detect outside of 8-15 μM?
2) What do you envision for iGEM?
3) Would it be more helpful to engineer a pathway with standard systems instead of using zinc biosensor?
   ↳ proof of concept for precision vs. dynamic engineering?
4) Can you provide an analogy to clarify the differences between precision and dynamic engineering?
5) Could you clarify which colors correlate to which levels of zinc? There was an error in the press release.
6) Is there a kill switch?
7) How exclusive is the biosensor to zinc?

Notes: Skype Interview

- The system has no killswitch
  ↳ However, we can use precision metabolic engineering to create one.
- **Degradation Tags** - adding these affects (positively, for the most part) how quickly you can move from one state to another.
- **Developing a Biosensor:**
  - You need something that can sense whatever you are trying to sense
    ↳ Ex: Transcriptional Regulatory Mechanism / Quorum Sensing techniques
  - ↳ **Challenges:**
    1) You need to ensure that your sensing mechanism is exclusive, meaning that it detects ONLY what you want it to detect.
    2) Time - Sometimes, it takes a whole day to get your desired response.
  - You also need a well designed and elegant construct.

Possible Solution:
- The use of "catalysts," especially pigment producing enzymes can help resolve this difficulty.
  → A catalyst (enzyme) that can expedite transcription would be ideal.

- **Inducer:** A chemical, which, when added, will bind to a molecule and invoke a certain behavior (in a different way).
  ↳ Standard Inducible Promoters (Ex: LacI) → Chromoproteins → Ex: TS Purple

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Chromoproteins RBS, Degration Tags:

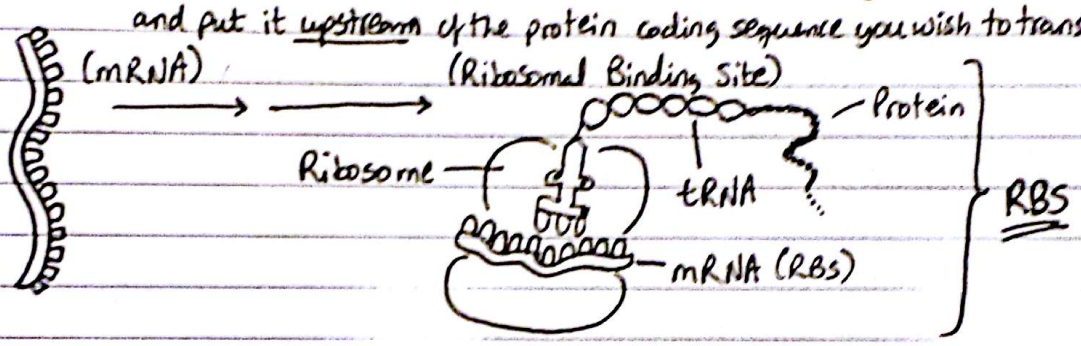
2/4/16

- **Reporters:**
- In synthetic biology, reporters are often used as a mechanism to display information.
  Ex: If you design a biosensor system that is sensitive to substrate A, you ideally want a way of displaying those results without needing an additional assay.
  ↳ - Fluorescent proteins cause a cell to fluoresce when excited with light of a particular wavelength
  - Luciferases cause a cell to catalyze a reaction that produces light.
  - Enzymes such as β-Galactosidase convert a substrate to a colored product.
- **There are many different types of reporters:**
  * 1) Chromoproteins
  2) Fluorescent proteins (ex: GFP/RFP)
  3) Luciferase proteins
  4) Enzymes that produce colored substrates.
- **Measurement:**
  - There are several different ways to measure or quantify a reporter depending on the particular reporter and what kind of characterization data is desired.
  - **Microscopy** - is useful for obtaining both spatial and temporal information on reporter activity, particularly at the single cell level.
  - **Flow Cytometers** - are suited for measuring distribution in reporter activity across a large population of cells.
  - **Plate Readers** - are generally best for taking population average measurements of many different samples over time.

- **Chromoproteins:**
  - Chromoproteins are often responsible for coloration in corals or sea anemones.
  - The expressed pigment can be seen by the naked eye without any external tool, making very useful proteins.
  - In short, they are a collection of reporter genes visible to the naked eye.
  - **Function:** to quantify the level of induction and amount of protein produced.
    Ex: tsPurple, scOrange, asPink

2/4/16

- **Ribosomal Binding Site:**
  - A ribosomal binding site (RBS) is an RNA sequence found in mRNA to which ribosomes can bind and initiate translation.
  - Translation Initiation in bacteria almost always requires both an RBS sequence and a start codon
    - ↳ In the registry, protein coding sequences begin with the start codon. So, if you want to build a BioBrick system that produces a protein, you need to pick an RBS part and put it upstream of the protein coding sequence you wish to translate.



- **Degradation Tags:**
  - Deg. Tags are short peptide sequences that mark a protein for degradation by the cell's protein recycling machinery. In doing so, the deg. tag effectively decreases the protein's half life or the typical length of time that a protein, once translated, will exist in a cell.
  - Deg. Tags decrease the [protein] in the cell.

- **Example:** Often times, a broken ("truncated") mRNA strand will be translated at ribosomes. This mRNA strand is broken in the sense that it does not have a normal termination codon and therefore the ribosome cannot detach from the defective mRNA.
  A special type of RNA known as "ssRNA" rescues the ribosome by adding an 11 codon degradation tag followed by a stop codon. This allows the ribosome to break free and continue functioning. This tag attached by the ssRNA is known as the ssRNA tag.
  - Ex: LAA and DAS

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- Constructs:
- 3 Main Repressible/Inducible promoters systems (Rep-Prom):
  1) LacI - pLac
  2) TetR - pTet
  3) AraC - pBad
  } All are found in registry.
- There are 2 different ways to approach the design of constructs:

① Production is fully repressed in "off" state and then turned "on" to a specific level upon addition of an inducer.:
- Have a chromoprotein under control of an inducible promoter, and upon induction measure the steady state induced level of production and/or measure how fast the protein is produced.
- Changing RBS from strong to weak one
- Adding degredation tags
  } will decrease the amt. protein produced & increase time req'd to achieve certain level of production.
- Main things to characterize in this:
  - how different modifications like ribosomal binding sites and protein deg. tags change level of induction.

Little more tricky.

② Production is "on" initially and then turned "off" upon addition of an inducer:
- Use 2 different inducible promoters to create a sort of "switch" such that the chromoprotein is initially expressed and then turned off.
  ↳ You would have to pick which of the 2 would drive expression of the chromoprotein by choosing the one that is "on" in the default state w/o addition of any inducer.

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Gene Expression in Organisms

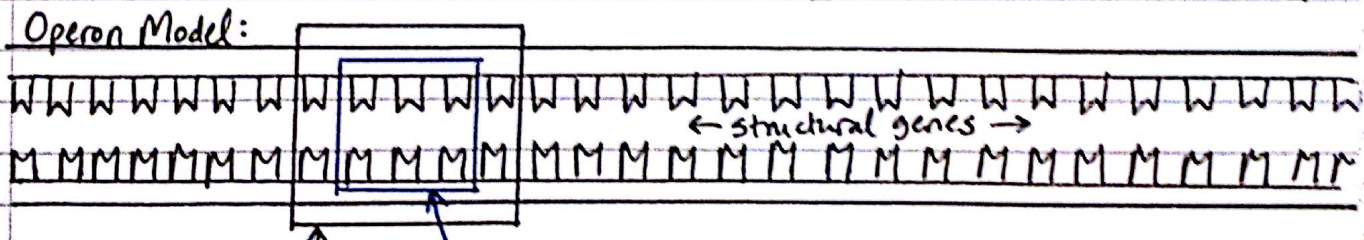
2/18/16

- **Gene Expression in Prokaryotes:**
  - Gene expression is how genes are turned ON or OFF. In Prokaryotes, it is rather simple with only a few controls
  - **Operons**: Sections of DNA that consist of one or more genes and their controlling elements.
    - In the middle of an operon are **structural genes**: sections that actually code for one or more mRNA molecules, which ultimately code for one or more proteins.
    - Inside the operon is a **promoter region** that "turns on" the prokaryotic gene. RNA polymerase must attach to this promoter so that it can start synthesizing the mRNA molecule.
    - A region called the **operator** can "turn OFF" the whole operon. If a regulatory molecule, such as a **repressor**, attaches at the operator, the operon is turned OFF because RNA polymerase is blocked from continuing down the strand.

* Blocking or unblocking the operator is how bacterial cells produce only certain proteins at certain times.

Regional Classification:
1) Operon
↳ 2) Operator
↳ Promoter

Operon Model:



(Example of Prok. Gene Exp. →)

- **Gene Expression in Eukaryotes:**
  - Unlike prokaryotes, eukaryotes do not have operators. They have:
    1) **Enhancers**: Sections of DNA that increase the expression of a gene.
    2) **Silencer**: Sections of DNA that decrease the expression of a gene.
  - Basically, eukaryotic genes are always "on," but expressed at very low levels. Expression is either increased or decreased as molecules interact w/enhancer or silencer regions.

BOOK NO. PROJECT

- Prokaryotic Gene Expression Example:

The lac operon, first discovered in E. Coli, is one of the best studied examples of an operator containing gene complex in biology.
It is a group of three genes that controls the metabolism of lactose, a sugar. Because E. Coli prefers glucose over lactose as a source of sugar, the lac operon functions to shut down the pathways that metabolize lactose when glucose is present in the cell, so that the organism can focus on its preferred energy source.

- Prokaryotic Gene Regulation:

- In proks, metabolism may be controlled in one of 2 ways:
  1) Enzyme Inhibition - slows down enzymatic processes and provides effective control of the metabolic pathway.
  2) Regulator Protein - This protein binds to the operator region of the operon. Through this approach, prokaryotes are able to more effectively control their metabolic pathways over time by regulation of the genes that produce enzymes necessary for different metabolic processes.

Ex: Repressors.

Gel Electrophoresis Lab

2/26/16

- **Introduction:**

The purpose of this activity is to demonstrate one of the underlying principles of biotechnology by performing gel electrophoresis on common biological reagents and macromolecules such as proteins and DNA. Gel Electrophoresis is an analytical method for the separation, identification and analysis of biological molecules, including DNA, RNA, and proteins, in an electrical field. Proteins with different sizes, shapes, and molecular charges move through the gel at different rates, with smaller molecules or fragments moving faster through the maze of microscopic pores. For example, a negatively charged protein migrates through a gel toward the positive electrode, which is called an anode. With that in mind, there are many pragmatic applications of this procedure, such as DNA fingerprinting. However, in this context, learning how to properly and successfully run a gel will apply to future researching endeavors for the Lambert iGEM team.

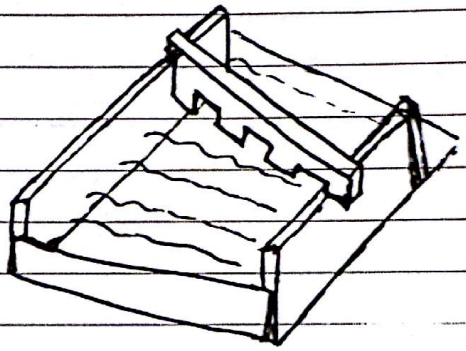
- **Materials:**

1) Agarose Gel
2) Methylene Blue Staining Solution (50mL)
3) TAE electrophoresis buffer
4) Distilled H_2O
5) Beakers (600mL) x2
6) Digital Micropipets
7) Electrophoresis Chamber with power supply
8) Marker
9) Paper Towels
10) Staining Tray
11) Thermometer
12) Biological Reagents/Samples (any protein/macromolecule/DNA)
13) Metric Ruler
14) Resealable bag (ex: Ziploc)
15) White Surface or sheet of paper
16) Lightbox or other light source (opt.)
17) Disposable Pipet Tips.

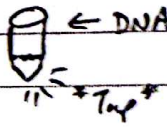
- Procedures: (Part I)

① Gently slide a prepared gel from a resealable bag into casting tray with wells toward cathode (−) end of the unit.
* Be careful not to break or crack the gel. If gel is damaged, do not use it! *

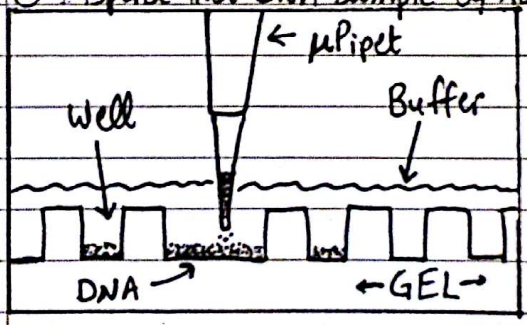
② After carefully positioning the gel and tray in electrophoresis chamber, pour enough electrophoresis buffer into the unit to submerge the entire gel 2-5 mm.



③ Shake the microcentrifuge tubes containing DNA samples and lightly tap the bottom of each tube to mix the contents.



④ Dispense first DNA Sample by holding pipet tip just inside the well. Be careful not to puncture the bottom or sides of the well.



⑤ Repeat ↻ Step 4 for remaining DNA samples.
* Use a clean pipet tip for each sample. *

(Part II):

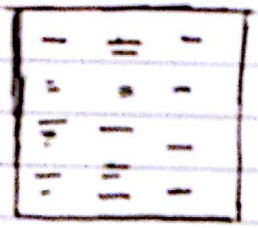
⑥ Place lid on electrophoresis chamber and turn it on.

⑦ Turn off the power when the first dye is 1 cm from (+) end of the gel. This may take 30 min − 2 hours.

⑧ When the power is off, place gel tray on a paper towel, being careful not to crack the gel.

(Part III):

(9) Slide gel off the tray and into the staining tray.



(10) Gently pour 40mL of Methylene Blue staining solution into staining tray.

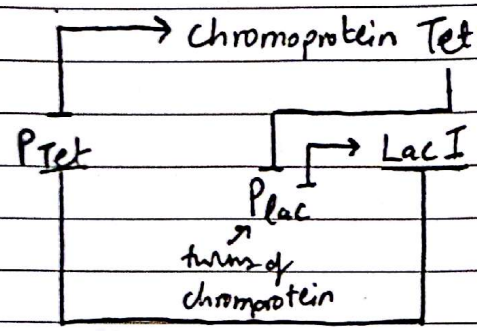
Allow gel to stain for at least 5-10min (L)

(11) Pour off stain into a beaker and then pour room temperature distilled H_2O into staining container. (DO NOT exceed 37°C) Then, destain for about 10 min.

(12) Pour off the H_2O into a waste beaker and repeat step 11 until DNA bands are visible.

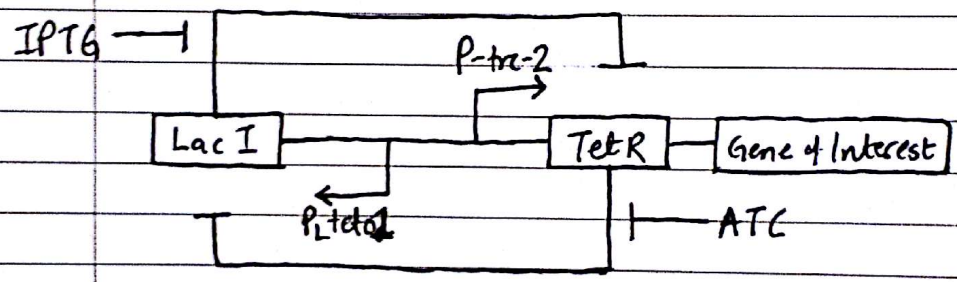
Genetic Circuits Notes

3/1/16



- **The Toggle Switch:**
- The Toggle Switch is a circuit consisting of two repressors:
  1) TetR
  2) LacI

  } Both mutually repress each other



- The LacI repressor is inhibiting the P-trc2 promoter → represses reporter
- The TetR repressor is inhibiting the P_L tet01 promoter → promotes reporter
- The ATC Inducer inhibits the expression of TetR repressor, therefore promoting the expression of P_L tet 01 promoter and consequently the LacI repressor.
- The IPTG inducer inhibits the expression of LacI repressor, therefore promoting expression of ~~TetR~~ Ptrc-2 promoter and consequently the gene of interest.

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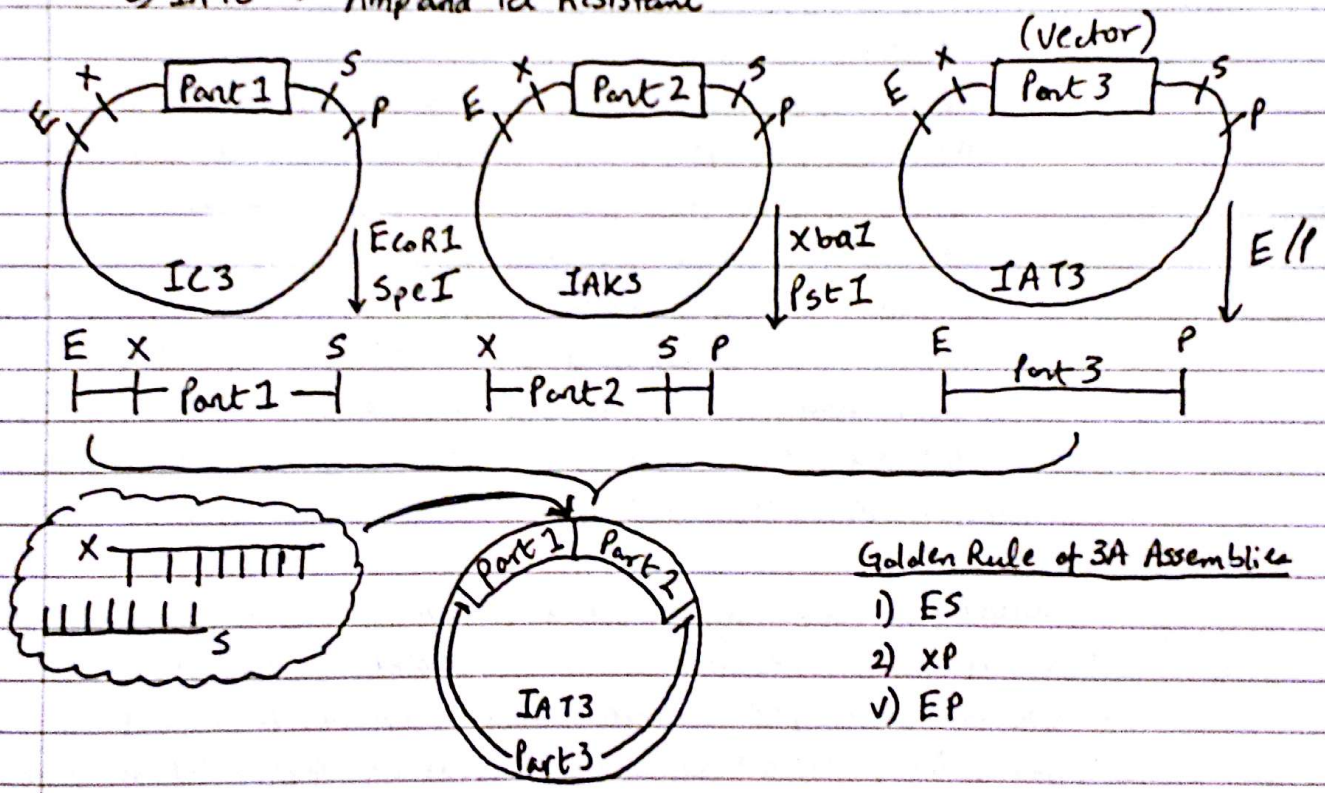
Ga Tech Notes on Assemblies

3/4/16

- There are 3 types of antibiotics that you use in your plates:
  1) Kanamycin (Kan)
  2) Chloramphenicol (Cam)
  3) Tetracycline (Tet)
  4) Ampicilin / Carbenicilin (Carb)
- There are also 3 types of Plasmids:
  1) IC3 → Cam Resistant
  2) IAK3 → Amp and Kan Resistant
  3) IAT3 → Amp and Tet Resistant



Golden Rule of 3A Assemblies

1) ES
2) XP
v) EP

BOOK NO. PROJECT

~~Project~~ Overview of ClpX & ClpP 3/23/16

Protein degradation plays a crucial role in maintaining homeostasis throughout the cell. When a protein is sentenced for destruction, it is tagged or somehow otherwise edited to notify proteases within the cell to degrade it. Perhaps the most well known and well studied protein degradation pathway is the ssRA-RNA tagging system in which ClpXP and ClpAP, two protease systems, are reported to play a crucial role.

According to studies conducted by numerous synthetic biology labs, ranging from University of California and Stanford to MIT, both ClpXP and ClpAP share the following characteristics:

1) Both identify ssRA tagged proteins and denature them into linear peptide strands.
2) The linear peptide strands created by denaturing by both proteases are fed into the same proteolytic subunit: ClpP. In other words, both ClpXP and ClpP share ClpP - a proteolytic subunit of both proteases.
3) Both are found in the cytoplasm of E. coli cells.
4) Both contribute to the degradation of proteins that contain an 11 peptide residue on the C-terminus of the substrate.

In other words, because both proteases degrade proteins in such an indistinguishable manner, there has been widespread controversy among the synthetic biology community over which protease, ClpXP or ClpAP, contributes more to the degradation of proteins.

With this in mind, the main purpose of this project is to investigate a clear difference between ClpXP and ClpAP. This will be accomplished by studying the time it takes for both ClpXP and ClpAP to degrade the chromoprotein, violacein. Furthermore, another side goal of this project is to characterize ClpXP and expand the already known information about it.

Moreover, in order to fully understand the construct (see page 17), the structure of ClpXP and ClpAP must be understood. Both ClpXP and ClpAP have a very similar structure; they are both protein complexes. In other words, they are a protein mechanism made up of different proteins that function together to accomplish a common goal. In this case, the protease system ClpXP is composed of the proteins ClpX and ClpP. According to recent studies, it has been revealed that ClpX is the protein that identifies the target protein and brings it back to the protein ClpP. Then, ClpP actually degrades the protein.

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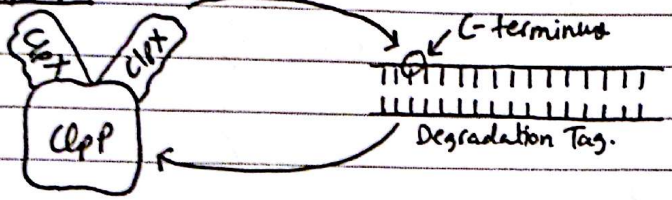
This same mechanism applies to ClpAP. Below are diagrams of what ClpXP and ClpAP look like. Note that these diagrams are only rough speculations of what the protease systems might look like.

ClpXP: *

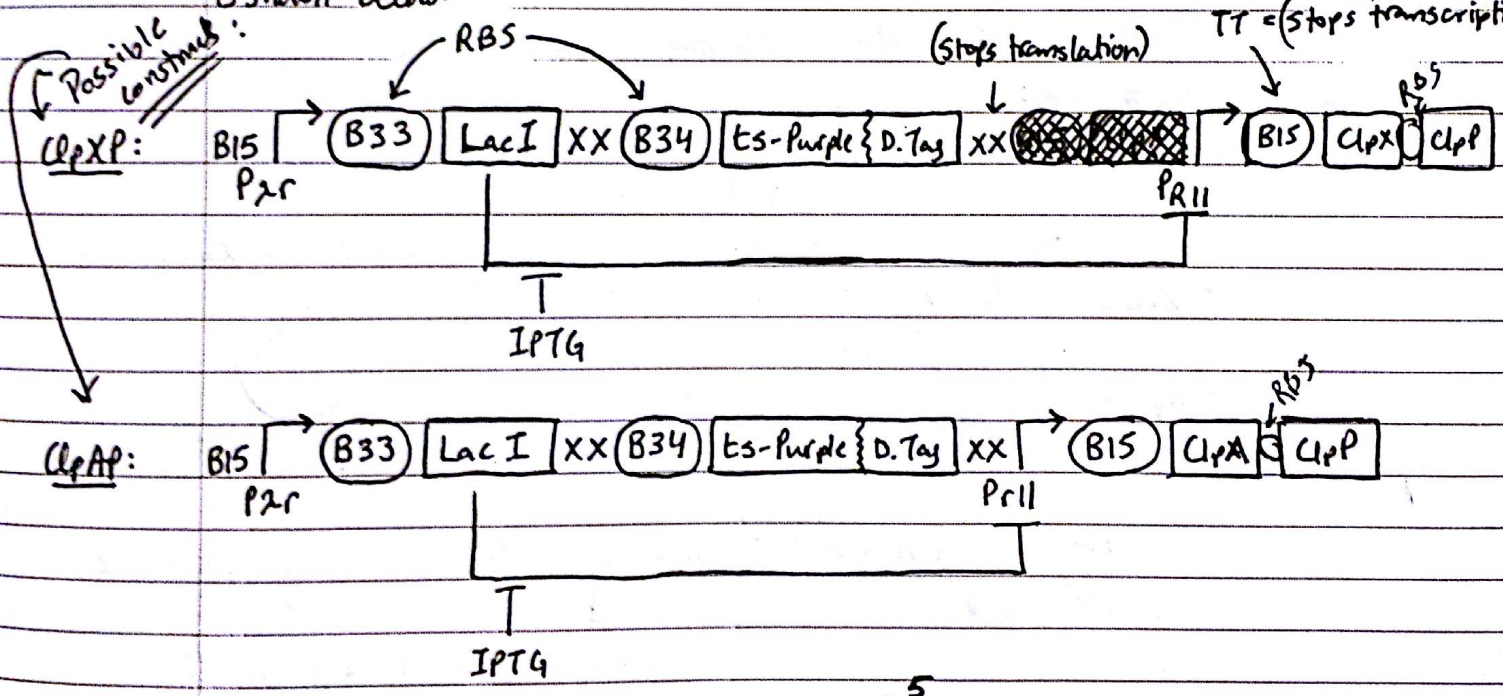


ClpX identifies deg. tag. on C-Terminus, then carries back to ClpP, which then degrades it.

* This same mechanism applies to ClpAP.

Finally, the constructs that will be used constructed for this project will be a repressible genetic circuit that will involve LacI, ts-Purple, and P2r/PrII promoters. The construct is shown below:

Possible constructs:



The 3A Assembly of this Construct is split into ~~5~~ 5 main parts:

1) Digestion
2) Ligation
3) Transformation
4) Screening
5) Liquid Culture/MiniPrep

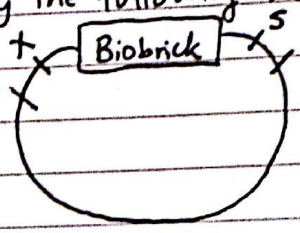
} Repeat 2x with Parts 1-2 and 3.

4/1/16

Assemblies

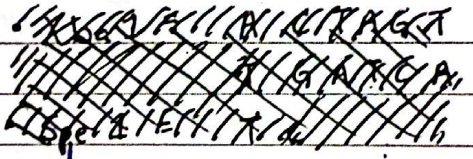
2A Assembly:

- A 2A Assembly (and subsequently a 3A assembly) is used to combine two different biobricks into one composite plasmid.
- Each biobrick is flanked by the following restriction sites:
  - E = EcoR1
  - X = Xba 1
  - S = Spe 1
  - P = Pst 1



- So, scientists can use these restriction sites to isolate a biobrick and combine it with another biobrick.
  - ↳ For example, if one cuts a biobrick plasmid at the E and P sites, one won't be able to close the plasmid upon itself.

However, something interesting happens when one cuts at the X and S sites:



- Xba 1 = T|CTAGA → CTAGA
  AGATC|T → T
- Spe 1 = A|CTAGT → A
  TGATC|A → TGATC

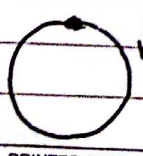
This is how Xba1 is cut

How Spe1 is cut

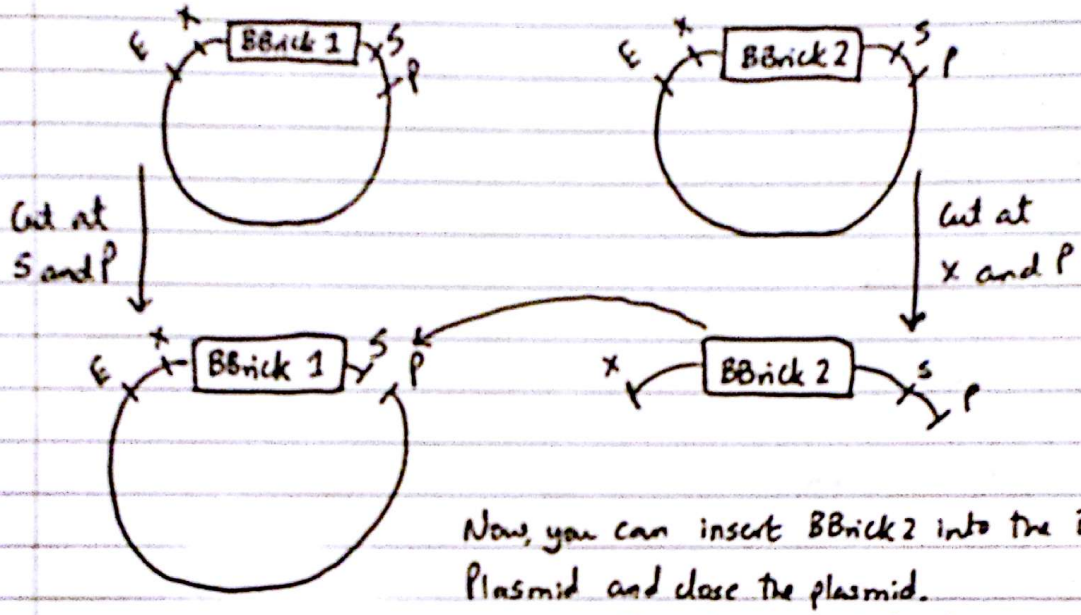
Xba1 site { CTAGA — T } Biobrick 1 { A — TGATC } Spe 1 site

loop it up (close the plasmid)

ACTAGA
TGATCT



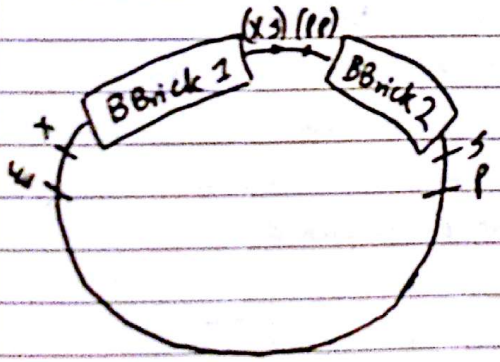
- So, in short, the Xba 1 site and Spe1 are able to bind together.
- With this in mind, this is how a 2A Assembly works:



Now, you can insert BBrick 2 into the BBrick 1 Plasmid and close the plasmid.

- The S site will bind with the X site as explained before.
- The P site (from #2) will bind with the P site (from #1).

Final Plasmid:



Summary of what binds with what:

- E · E
- X · X
- * - X · S *
- S · S
- P · P

Notice how in the final plasmid, the restriction sites E, X, S, and P still flank the new composite biobrick. You can use this to combine this new composite biobrick with another biobrick. This is the genius behind assembly.

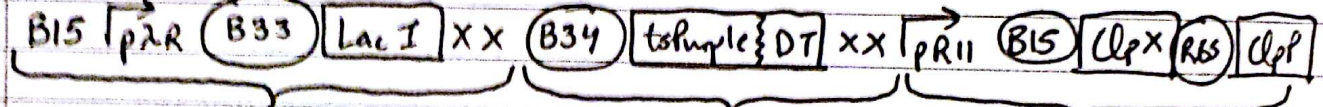
(Lol)
4/20/20

Procedure Overview
~~Detailed Procedure:~~

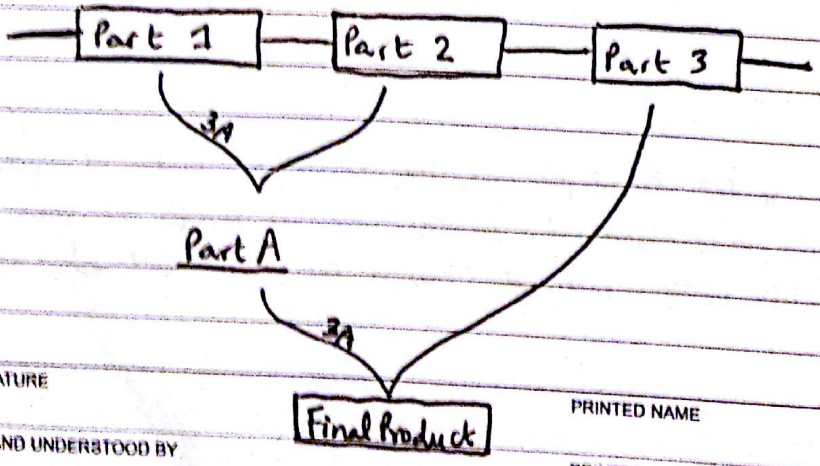
- **Part 1:**
  B15 → first XX Codon
- **Part 2:**
  B34 → second XX Codon
- **Part 3:**
  B15 → ClpP

- **Materials Needed**
  - Cam Plates
  - IC3 Plates
  - IAK3 Plates
  - Part 2 from Monica.

- **Procedure Overview:**



As shown above, the construct for this project will be divided up into three different parts. Part 1 extends from B15 to the first double x codon; Part 2 extends from B34 to the second double x codons; finally, part 3 extends from pR11 to ClpP. Parts 1 and 2 will be assembled through a 3A Assembly to create part A. Part A will then be assembled with Part 3 (through a 3A Assembly, of course) to produce the final, composite construct.



INVENTOR SIGNATURE
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DATE
DISCLOSED TO AND UNDERSTOOD BY
PRINTED NAME
DATE

Basic Workflow:

4/21/16

- Overview of 3A Assembly Protocol:
  1) Digestion
  2) Ligation
  3) Transformation
  4) Screening
  5) Liquid Culture
  6) Miniprep

1) Digestion:
- Rehydrate in 10μL of H_2O for 100ng/μL [].
- Digest E & S to ligate with 1C3

2.5 μL	DNA
2μL	EcoR1
1μL	Spe1
15.5 μL	H_2O

* See Google Drive for the detailed procedure!

ClpXP & Protein Degradation

5/1/16 → 6/29/16

- AAA+

AAA or AAA+ is an abbreviation for ATPases Associated with diverse cellular Activities. It is part of the family known as ATPases, ~~in which~~ of which there are numerous types, including V-ATPases, P-ATPases, F-ATPases and AAA ATPases (AAA+). The AAA ~~Subclasses~~ Subclass of ATPases forms rings, usually hexameric, with a central pore and it regenerates mechanical force by undergoing conformational changes during cycles of ATP binding and hydrolisis.

In all organisms, AAA ATPases are involved in processes as diverse as protein unfolding and degradation, peroxisome ~~degradation~~ biogenesis, bacteriochlorophyll biosynthesis, and DNA recombination, replication and repair. For example, both FtsH and ClpX families of AAA+ proteins ~~are~~ function to unfold proteins and direct them for proteolytic degradation.

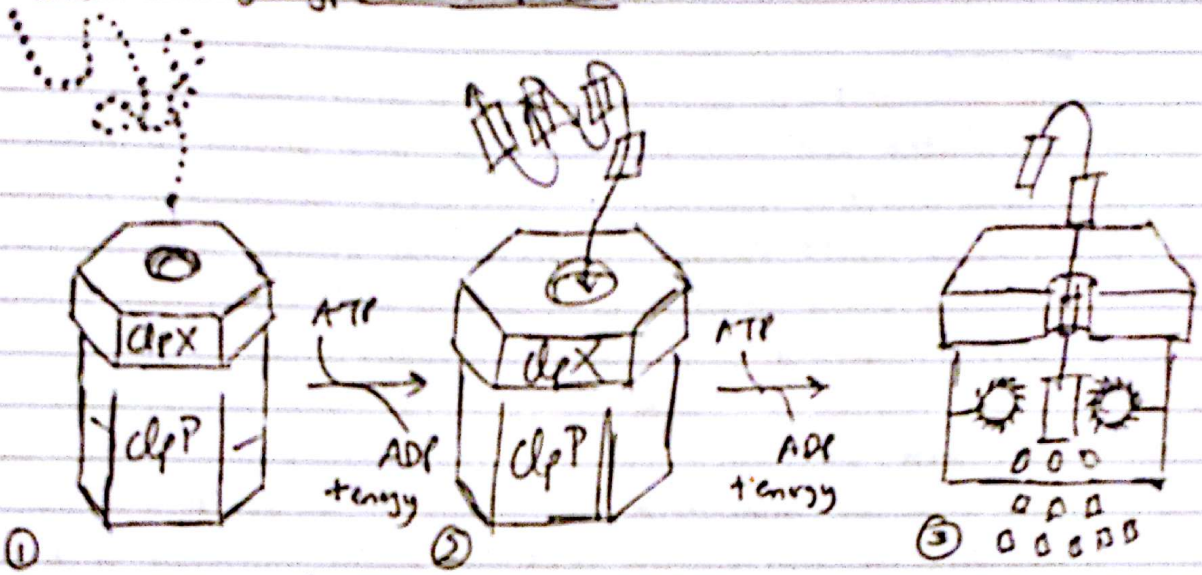
- ClpXP (from E. 66)

ClpXP is a AAA+ protease that uses the energy of ATP binding and hydrolisis to perform mechanical work during targeted protein degradation within cells. As mentioned before, AAA+ proteins utilize the energy of ATP binding and hydrolysis to perform the mechanical work required to power numerous biological reactions and processes

- In ClpXP:

i) ClpX recognizes unstructured peptide sequences (called tags or degrons, in this case: degradation tags) in protein substrate, and then proceeds to unfold stable tertiary structure in the protein. Finally, it spools or translocates the unfolded ~~protein~~ protein/~~pro~~ polypeptide chain into a sequestered proteolytic compartment in ClpP for degradation into small peptide fragments.

Degradation by a typical AAA+ Protease:



In an initial recognition step, a peptide tag in a protein substrate binds in the axial pore of the ClpX hexamer. In subsequent ATP dependent steps, ClpX unfolds the substrate and translocates the unfolded polypeptide into the degredation chamber of ClpP for proteolysis, where it is cleaved into small peptide fragments

- ATPAse (more Info:)

A class of enzymes that catalyze the decomposition of ATP into ADP and a free phosphate ion. This releases energy. During this dephosphorylation reaction, the enzyme harnesses the energy released to drive other chemical reactions that would otherwise not occur.

Dephosphorylation Reaction:

$$ATP \rightarrow ADP + PO_4^{3-} + Energy$$

SWITCH

6/2/16

1) Abstract:

make sure to indent in MPed version!

The concentration of proteins in a cell is determined by both the amount synthesized and amount degraded. Thus, protein degradation is a crucial aspect of maintaining intracellular equilibrium. A class of ATPases known as AAA+ Proteins involves a well known proteolysis mechanism known as ClpXP in which ClpX unfolds and translocates a tagged protein into a sequestered proteolytic compartment in ClpP.

We devised an ~~IPTG~~ inducible genetic construct in which ClpXP ~~with~~ degrades a chromoprotein upon induction by IPTG. The data will be ~~quantified~~ gathered using a device that can ~~capture~~ quantify the color of light reflected by the chromoprotein before and after induction. This will ultimately allow us to ~~see~~ measure the relative strength of degradation ~~by~~ and further characterize a well-known proteolysis mechanism. Our characterization of ClpXP will serve to enhance existing biological systems by fine tuning ~~existing~~ specific protein thresholds in their systems.